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Endotoxin challenge increases xanthine oxidase activity in cattle: effect of growth hormone and vitamin E treatment☆

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Abstract

In addition to its basic role in the metabolism of purine nucleotides, xanthine oxidoreductase (XOR) is involved in the generation of oxygen-derived free radicals and production and metabolic fate of nitric oxide (NO). Growth hormone (GH) and Vitamin E (E) have been shown previously to modify immune response to infection. Our objective was to determine in heifers the effect of endotoxin challenge (LPS; 3.0 μg/kg BW, i.v. bolus, *Escherichia coli* 055:B5) on xanthine oxidase (XO) activity in plasma and liver and the modification of this response by daily treatment with recombinant GH (0.1 mg/kg BW, i.m., for 12 days) or GH + E (E: mixed tocopherol, 1000 IU/heifer, i.m., for 5 days). In experiment 1, 16 heifers (348.7 ± 6.1 kg) were assigned to control (C, daily placebo injections), GH, or GH + E treatments and were challenged with two consecutive LPS injections (LPS1 and LPS2, 48 h apart). After LPS1, plasma XO activity increased 290% ($P < 0.001$) at 3 h, reached peak (430%) at 24 h and returned to basal level by 48 h after LPS2. XO responses (area under the time × activity curve, AUC) were greater after LPS1 than LPS2 ($P < 0.001$). Total plasma XO responses to LPS (AUC, LPS1 + LPS2) were augmented 55% ($P < 0.05$) over C with GH treatment but diminished to C responses in GH + E. There was a linear relationship ($r^2 = 0.605$, $P < 0.001$) between total response in plasma XO activity and plasma nitrate + nitrite concentration. In experiment 2, 24 heifers (346 ± 6 kg) were assigned to C or GH treatments and liver biopsy samples were obtained at 0, 3, 6, and 24 h after a single LPS challenge. Hepatic XO activities increased 63.3% ($P < 0.05$) 6 h after single LPS challenge and remained elevated at 24 h (100.1%, $P < 0.01$) but were not affected by GH treatment. Results indicate that LPS-induced increases in plasma XO activity could be amplified by previous GH treatment but attenuated by E administration. The data also suggest that E may be effective in controlling some mediators of

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immune response associated with increased production of NO via the effect on XO activity and its production of superoxide anion as well as uric acid.
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1. Introduction

The basic role of xanthine oxidoreductase (XOR) is to catalyze the hydroxylation of hypoxanthine and xanthine to uric acid (UA) in the latter stages of purine catabolism [1]. In mammals, XOR occurs in two interconvertible forms, xanthine dehydrogenase (XDH; EC 1.1.1.204) and xanthine oxidase (XO; EC 1.1.3.22). The former solely uses NAD^+ as an electron acceptor yielding NADPH whereas the latter exclusively uses O_2 yielding reactive oxygen species O_2^- and H_2O_2 as reaction by-products [2,3]. XOR is widely distributed among animal species and tissues with the highest activity in liver and intestine [1]. In cytosol, XOR typically exists as XDH but can be reversibly converted to XO by thiol oxidation or irreversibly converted to XO through proteolytic cleavage [4]. In extracellular spaces, including serum, the XO form predominates [5].

In the last decade, much interest has been focused on the possible role of XO in initiating and modulating the immune and metabolic response to infection [6]. Xanthine oxidase is involved (a) in the generation of UA, which can serve as a general antioxidant [7], (b) in the production of oxygen-associated molecular reactants (O_2^-, H_2O_2, $ONOO^-$), which can initiate oxidative cell damage and organ failure [8], and (c) in the generation and metabolic fate of nitric oxide (NO), an important component and regulator of the immune response to infection [9] and circulatory and neuronal functions [10]. Recently, it was shown that XO is responsible for H_2O_2-mediated killing of invading trypanosomes in *Trypanosoma brucei*-resistant Cape buffaloes [3].

Increased XO activity in liver has been reported in mice during viral, bacterial and protozoal infection or with Ehrlich ascitic carcinoma [11]. Increased expression of XDH/XO at both the activity and gene levels was observed in vitro after treatment with inflammatory cytokines [12] and in vivo after LPS administration [13].

The development of intervention strategies to limit the morbidity and mortality associated with infection and sepsis is an ongoing challenge to human and veterinary medicine. Key to targeting a therapy is the identification of critical control points and regulatory effectors in the associated biochemical pathways at which changes in molecular interactions result in improved clinical outcome. The use of growth hormone (GH) to manage patient response to infection has received attention because of the ability of GH to modulate immune function as well as promote anabolic activity. To this end some clinical researchers had reviewed the utility of GH in septic situations and concluded that the adjunct is with beneficial merit [14]. However, intriguing in concept, GH use as an adjunct therapy in sepsis is equivocal and has lately been criticized. Growth hormone treatment during certain stages of the catabolic conditions of sepsis has been shown to increase mortality and morbidity of critically ill patients [15]. Also in rats, GH administration potentiated the in vivo biological activities of LPS [16]. We have previously shown that GH administration augments and Vitamin E (E)

attenuates NO production after LPS challenge in heifers [17]. Among its beneficial actions, E has been indicated as the major antioxidant that prevents the propagation of free radical damage in biological membranes [18].

To date there are no reports or studies in the literature that address the simultaneous change in XO activity and NO production as affected by GH treatment prior to the onset of an oxidative immune stress. The purpose of the present study was to investigate in heifers the effect of LPS challenge on plasma and liver XO activity and the modification of this response by daily treatment with recombinant GH and Vitamin E.

2. Materials and methods

2.1. Animals and experimental design

All experiments were performed in accordance with approval of the Animal Care and Use Committee at the USDA Agricultural Research Service (Beltsville, MD, USA, Protocol #01-021). In a preliminary experiment, six crossbred beef heifers (362 ± 11 kg BW), were challenged with a single dose of LPS (as described below for experiments 1 and 2) or saline ($n = 3$ per group) and jugular blood samples were obtained at 0, 1, 2, 3, 4, 6, 8, 24, 48, and 96 h relative to LPS or saline injection. The purpose of this preliminary experiment was to determine the pattern of changes in plasma XO activity after a single LPS challenge.

In experiment 1, 16 crossbred beef heifers (348 ± 6 kg BW) were fed individually a forage-concentrate diet (17.8% CP) to appetite, and synchronized to the diestrus stage of the estrous cycle with the suggested two-injection protocol for the $PGF_{2\alpha}$ analog dinaprost tromethamine (Lutalyse®, Upjohn Company, Kalamazoo, MI). Heifers were assigned to control (C, daily corn oil and saline-bicarbonate injections), recombinant GH (0.1 mg/kg BW, i.m.; Monsanto Company, St. Louis, MO), or GH + Vitamin E (E, 1000 IU per day, i.m., mixed tocopherol isomers, Sigma, St. Louis, MO) treatments. Heifers were treated with GH for 12 days and with E for 5 days before LPS challenge. For stability, E was maintained at −20 °C in an argon-purged atmosphere after initial vial opening. All heifers were challenged with LPS (3.0 μg/kg BW, i.v. bolus via indwelling jugular catheters, *E. coli* 055:B5) 8 days after the last injection of Lutalyse® (LPS1) and again 48 h later (LPS2). For each challenge jugular blood samples were obtained at 0, 1, 2, 3, 4, 6, 8, 24, and 48 h relative to LPS injection. Blood plasma samples were stored at −20 °C until assayed.

In experiment 2, 24 heifers (346 ± 6 kg BW) were assigned, in a 2 × 4 factorial design, to C or GH treatments and four liver biopsy times. Diet, Lutalyse® injections, and C and GH treatments were the same as in experiment 1. All heifers were challenged with LPS (3.0 μg/kg BW, i.v. bolus) and liver biopsy samples were obtained, from respective groups, at 0, 3, 6, and 24 h after LPS injection using a commercial biopsy instrument (True-Cut; Baxter, Columbia, MD) as previously described [19]. Biopsy samples were immediately frozen in liquid nitrogen and stored at −80 °C until assayed. Jugular blood samples were obtained at 0, 1, 3, 6, and 24 h relative to LPS injection. Blood plasma samples were stored at −20 °C until assayed.

2.2. Xanthine oxidase determination

Plasma XO activities were determined in duplicate using Amplex™ Red Xanthine/Xanthine Oxidase Assay Kit (A-22182, Molecular Probes, Eugene, OR) validated for bovine plasma. Before the assay, plasma samples were diluted 1:10 (v/v) in 0.1 M Tris–HCl, pH 7.5. For XO assay in liver, biopsy samples were homogenized in ice-cold 0.1 M Tris–HCl buffer (1:5, w/v) using a Polytron homogenizer (Brinkman Instruments Inc., Westbury, NY, USA). Homogenizing buffer (pH 7.5) contained 0.1 ml of protease inhibitor cocktail (Sigma, St. Louis, MO) per 1 ml of buffer. After centrifugation at 100,000 × *g* for 30 min, the supernatant was diluted 1:100 (v/v) with 0.1 M Tris–HCl and directly used in the assay mixture. Protein concentration in liver homogenates was determined with a bicinchoninic acid reagent protocol (Pierce Chemical Co., Rockford, IL, USA) and BSA used as a standard.

2.3. Plasma nitrate + nitrite determination

The stable end-products of the NO pathway and markers of NO production, $NO_2^- + NO_3^-$ (NO_x), were measured using Griess reaction after enzymatic conversion of plasma NO_2^- to NO_3^- with nitrate reductase from *Aspergillus* species [19].

2.4. Uric acid determination

Plasma uric acid (UA) concentrations were determined in duplicate using Amplex™ Red Uric Acid/Uricase Assay Kit (A-22181, Molecular Probes, Eugene, OR) validated for bovine plasma. Before the assay, plasma samples were diluted 1:4 (v/v) in 0.1 M Tris–HCl, pH 7.5.

2.5. Statistical analysis

Response to LPS challenge for plasma XO or NO_x was calculated as area under the time × concentration curve (AUC) with baseline subtracted (concentration at 0 h). The AUC for XO and NO_x response was calculated, respectively, over the 96 and 72 h period after LPS1 challenge. Changes in XO plasma activities were analyzed using the MIXED procedure of SAS [20] with treatment (C, GH, GH+E) and time after LPS challenge as fixed effects. Time after LPS challenge was considered repeated on the same heifer, which was nested within the treatment. When significant effects were detected ($P < 0.05$), differences between means were further separated by the ESTIMATE option of SAS. Response data (AUC) for plasma XO and NO_x were analyzed using one-way ANOVA for each LPS challenge. Changes in plasma and hepatic XO activity and plasma UA concentration in experiment 2 were analyzed using two-way ANOVA. Data are presented as least squares means ± S.E.M.

3. Results

Administration of LPS to heifers (LPS1) resulted in transient signs of systemic illness characterized by an increase in rectal temperature for 4 to 6 h (1.31 ± 0.11 °C increment

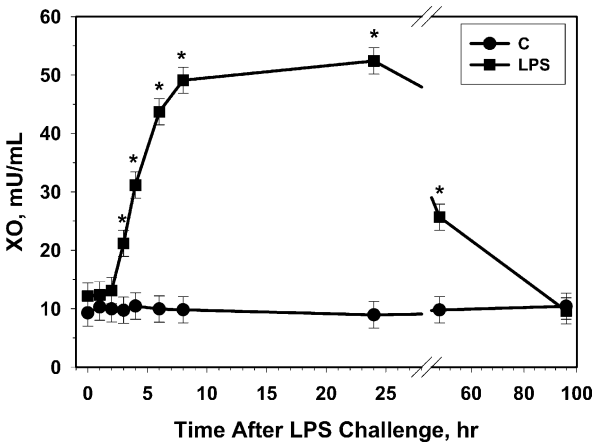

Fig. 1. Response pattern in plasma activity of xanthine oxidase (XO) in heifers following single challenge with saline (C) or endotoxin (LPS; 3 μg/kg BW, i.v.). Data represent least square means ± S.E.M. ($n = 3$). $^{*}P < 0.01$ between C and LPS at the same time.

from base temperature at 0 h to peak temperature at 2 h, $P < 0.001$), a short period of labored breathing, slight coughing that dissipated within 3 h after LPS, mild diarrhea and lethargy. These signs were not apparent 8 h after LPS. However, no significant differences in the magnitude of the clinical signs were observed between treatment groups. All clinical responses, except for rectal temperature increases ($1.15 \pm 0.10\,^{\circ}C$, between 0 and 2 h, $P < 0.001$), were less pronounced during the second LPS challenge (LPS2), indicating the development of tolerance to repeated LPS challenges.

Response pattern in plasma activity of XO in heifers following challenge with single bolus injection of LPS is presented in Fig. 1. Compared with the activities observed in control heifers injected with saline, single LPS challenge increased plasma XO activity after 3 h ($P < 0.01$). The highest activities (~5-fold increase; $P < 0.01$) were attained between 8 and 24 h after LPS and returned to baseline by 96 h. The absence of a significant change in plasma XO activities in control heifers indicated that animal management and multiple blood collections during experimental period did not affect plasma XO activity. Effects of GH or GH + E treatment on plasma XO activities after two consecutive LPS challenges (LPS1 and LPS2) separated by 48 h are shown in Fig. 2. Compared to initial values at time 0 h, XO activities increased ($P < 0.01$) in all experimental groups at 4, 8, 24 and 48 h after LPS1. The subsequent LPS challenge at 48 h (LPS2) did not cause any additional increase in plasma XO but maintained higher than 0-h activities at 52, 54, 72 ($P < 0.01$ versus 0 h), and 96 h ($P < 0.05$) after LPS1. Heifers pretreated with GH had higher plasma XO activity than C animals at 4 h ($P < 0.05$), 8 and 24 h ($P < 0.01$) after LPS1 and tended to have higher activity ($P < 0.1$) at 4 h after LPS2 (i.e. 52 h after LPS1). Plasma XO activities after two consecutive LPS challenges did not differ between C and GH + E heifers.

Plasma XO responses to LPS challenges, measured as area under the time × activity curve (AUC) are shown in Fig. 3. In all experimental groups, plasma XO responses were lower after LPS2 than after LPS1 ($P < 0.001$). Compared to C heifers, plasma XO responses to

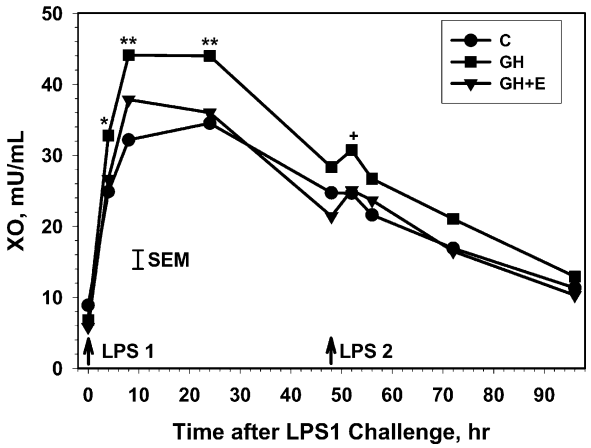

Fig. 2. Effect of growth hormone (GH) or GH + Vitamin E (GH + E) treatment on plasma xanthine oxidase (XO) activity after two endotoxin challenges (LPS1 and LPS2; 3 μg/kg BW, i.v.) separated by 48 h. GH (0.1 mg/kg BW) and E (1000 IU per heifer) were injected (i.m.) daily for 12 and 5 days, respectively. Control heifers (C) received placebo treatment (corn oil and saline-bicarbonate daily injections) and were challenged twice with LPS. Data represent least square means ($n = 5$ or 6 per group). $^{+}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. C at the same time.

LPS1 and LPS2 were higher ($P < 0.05$) and more prolonged ($P < 0.05$) in heifers pretreated with GH and these differences were evident whether AUC was calculated for each challenge separately or for both challenges combined (LPS1 + LPS2). In GH + E heifers, total plasma XO response to both LPS challenges did not differ from that observed in C heifers.

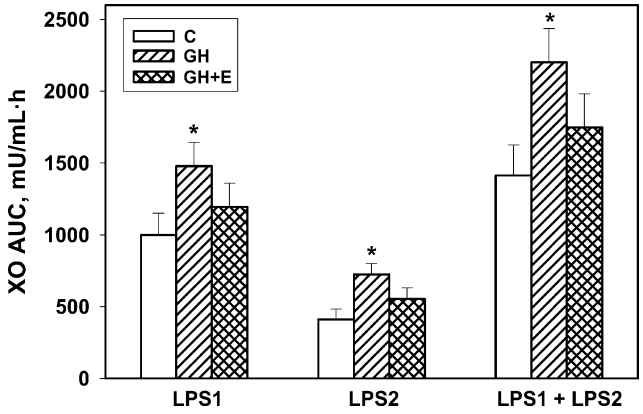

Fig. 3. Effect of growth hormone (GH) or GH + Vitamin E (GH + E) treatment on plasma xanthine oxidase (XO) response (area under the time × activity curve, AUC) to two endotoxin challenges (LPS1 and LPS2; 3 μg/kg BW, i.v.) separated by 48 h. AUC was calculated over 48 h period after each LPS challenge (LPS1 and LPS2) and for both challenges combined (LPS1 + LPS2). GH (0.1 mg/kg BW) and E (1000 IU per heifer) were injected (i.m.) daily for 12 and 5 days, respectively. Control heifers (C) received placebo treatment (corn oil and saline-bicarbonate daily injections) and were challenged twice with LPS. Data represent least square means ± S.E.M. ($n = 5$ or 6 per group). $^{*}P < 0.05$ vs. C in respective challenge.

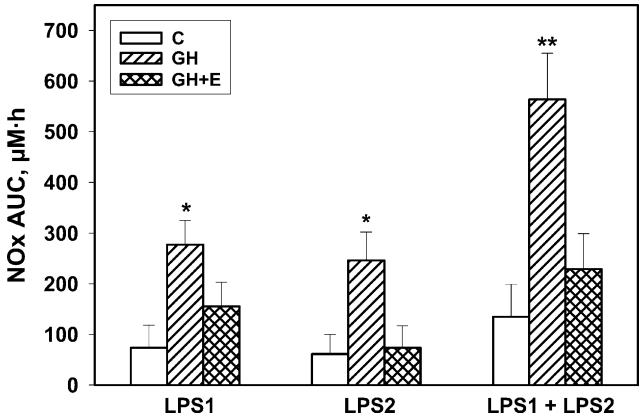

Fig. 4. Effect of growth hormone (GH) or GH + Vitamin E (GH + E) treatment on plasma nitrite + nitrate (NO_x) response (area under the time × concentration curve, AUC) to two endotoxin challenges (LPS1 and LPS2; 3 μg/kg BW, i.v.) separated by 48 h. AUC was calculated over 48 h period after LPS1 and 24 h period after LPS2 and for both challenges combined (LPS1 + LPS2). GH (0.1 mg/kg BW) and E (1000 IU per heifer) were injected (i.m.) daily for 12 and 5 days, respectively. Control heifers (C) received placebo treatment (corn oil and saline-bicarbonate daily injections) and were challenged twice with LPS. Data represent least square means ± S.E.M. ($n = 5$ or 6 per group). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. C in respective challenge.

Fig. 4 summarizes the effect of GH or GH + E treatment on plasma nitrate + nitrite responses (as AUC) to two consecutive LPS challenges. A preliminary overview of the NO_x plasma concentration data were published previously by Elsasser et al. [17]. Compared to C group, heifers pretreated with GH had greater plasma NO_x responses to LPS1 ($P < 0.05$)

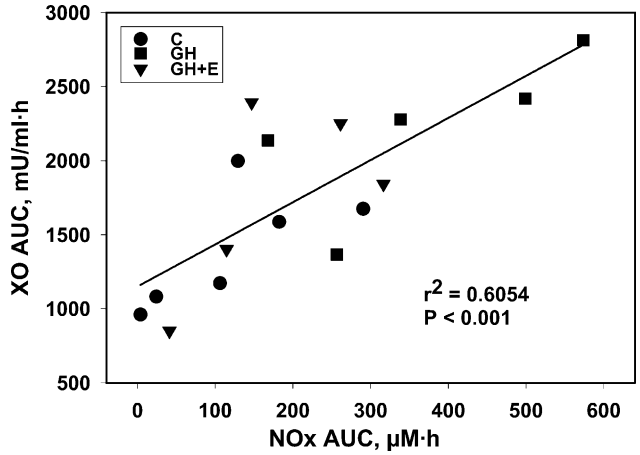

Fig. 5. Relationship between response in plasma nitrate + nitrite (NO_x) concentration and plasma xanthine oxidase (XO) activity after two consecutive endotoxin challenges (LPS1 and LPS2; 3 μg/kg BW) separated by 48 h. Response was calculated as combined (LPS1 + LPS2) area under the time × concentration curve (AUC) within 72 h and 96 h after LPS1 challenge for NO_x and XO, respectively.

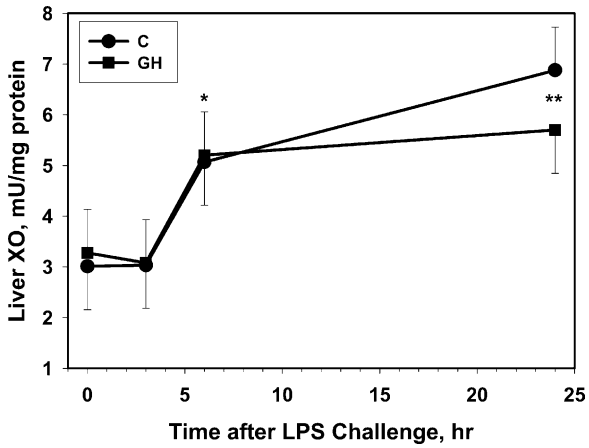

Fig. 6. Effect of endotoxin challenge (LPS, bolus, 3 μg/kg BW, i.v.) on hepatic xanthine oxidase activity (XO) in control (C) and GH-treated (GH) heifers. GH (0.1 mg/kg BW, i.m.) was injected daily for 12 days. C heifers received placebo treatment (saline-bicarbonate daily injections). Activities (mU) are expressed per mg of protein in the 100K × *g* supernatant of liver homogenate. Data represent least square means ± S.E.M. ($n = 3$). Because no differences were observed between control (C) and GH-treated heifers, differences vs. time 0 ($^{*}P < 0.05$, $^{**}P < 0.01$) were calculated for combined data (C + GH).

and LPS2 ($P < 0.05$). Also, combined total plasma NO_x response for LPS1 and LPS2 was greater in GH than C group ($P < 0.01$). In GH + E heifers, total plasma NO_x response to both LPS challenges did not differ from that observed in C heifers. There was a linear relationship ($r^2 = 0.6054$; $P < 0.001$) between total response (AUC, LPS1 + LPS2) in plasma XO activity and plasma NO_x concentration (Fig. 5).

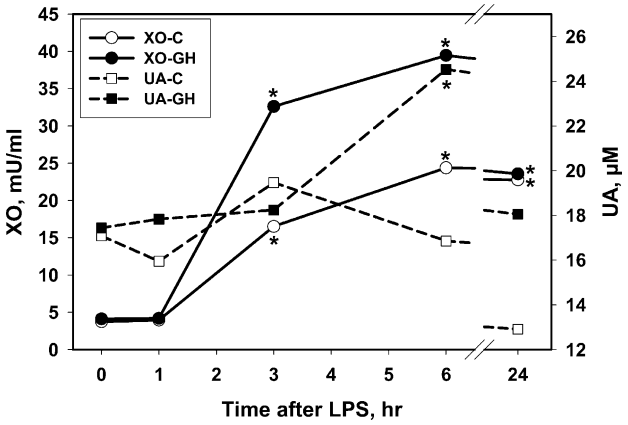

Fig. 7. Effect of endotoxin challenge (LPS, bolus, 3 μg/kg BW, i.v.) on plasma xanthine oxidase activity (XO) and uric acid (UA) concentration in control (C) and GH-treated (GH) heifers. GH (0.1 mg/kg BW, i.m.) was injected daily for 12 days. C heifers received placebo treatment (saline-bicarbonate daily injections). Data represent least square means ($n = 3$; pooled S.E.M. for XO = 2.92 mU/ml and for UA = 1.62 μM). Plasma XO and UA were affected ($P < 0.05$) by GH treatment. $^{*}P < 0.01$ vs. time 0 in respective treatment group.

Changes in hepatic XO activity after single LPS challenge are shown in Fig. 6. Hepatic XO activity was increased at 6 h after LPS challenge ($P < 0.05$) and remained elevated at the 24 h biopsy period ($P < 0.01$). GH treatment did not affect the basal hepatic XO activity (time 0 h) and did not modify the increased XO activity after LPS challenge (time 6 and 24 h).

Fig. 7 summarizes the effect of single LPS challenge (experiment 2) on plasma XO activity and plasma UA concentration in C and GH-treated heifers. Compared to initial values, plasma XO activities were increased in both experimental groups at 3 h after LPS challenge ($P < 0.01$) and remained elevated at 24 h ($P < 0.01$). However, plasma XO activities were higher ($P < 0.05$) in GH than in C heifers at 3 and 6 h after LPS challenge. Plasma UA concentrations were not affected by LPS challenge in C heifers but increased ($P < 0.01$), compared to initial values at 0 h, in GH-treated heifers 6 h after LPS administration. Plasma UA concentrations were higher in GH than C heifers 6 and 24 h after single LPS challenge ($P < 0.05$).

4. Discussion

The data presented here indicate that GH administration increases plasma activity of XO and plasma NO_x concentrations following a mild challenge with bacterial LPS. The observed response was modulated downward by pre-treatment of subjects with Vitamin E. While responses to the second administration of LPS indicated that tolerance was maintained, the extraordinary length of the XO response following the first LPS challenge indicated a potential for lasting physiological response long after the initiating acute response was over and still greater in the GH-treated animals. Plasma UA concentrations were also increased after LPS challenge in animals treated for 12 days with GH. When calculated as a function of the area under the activity-by-time response curve, GH treatment increased both the magnitude of the XO increase as well as the duration of increase. The temporal nature of the change in plasma XO activity was commensurate with measured increases in plasma NO_x, validated estimates of NO production [19]. An interesting and possible physiologically important feature of the XO-UA responses was that the first time point where the increase in plasma XO activity occurred was 3 h earlier than a time point where UA was found to be significantly elevated. This period of temporal discordance could provide a window of opportunity through which increased enzymatic capacities of XO are unchecked by its end-product feedback modulator, UA [21]. Furthermore, coordinated increases in NO production afford the opportunity for NO/superoxide anion interactions that could result in the production of a myriad of reactive oxynitrogen intermediates as well as perturbations in NO signal transduction processes throughout the body. The cellular compartmentalization of reactive oxygen species generation has been aligned with both beneficial function of neutrophils and macrophages, i.e. respiratory burst activity without tissue destruction [22] as well as pathological consequences associated with cytokine-driven superoxide anion generation in mitochondria and its role in apoptosis and cell death [23].

Xanthine oxidase, as a generating source of superoxide anion as well as H_2O_2 comprises a rather unique free radical generator because of its capacity to generate its reactants not only intracellularly but also extracellularly. The extracellular generation of reactive oxy-

gen species by XO is temporally commensurate with the significant increases in plasma or serum XO activity, as well as XO gene transcription [24] that typically occur with the defined stresses of parasitism [3], endotoxemia [25], and viral infection [26]. The importance of XO released into the circulation is reflected in XO-mediated oxidation and change in function of serum proteins and lipids [27] and heparin-ligand-mediated rebinding of XO to the extracellular plasma membrane matrix of vascular endothelial cells [28] with accompanying membrane oxidative stress [29]. An additional factor contributing to increased morbidity of host response is an XO-free radical-mediated induction of the complement cascade leading to the formation of multimeric complement C9 as a critical terminal transmembrane pore complex of the membrane attack pathway [30]. Some of the potential adverse effects of increased XO activity in serum (e.g. circulating lipid oxidation) are moderated in part by the availability of serum protein thiol groups to scavenge and neutralize O_2^- as well as H_2O_2 [27].

While the capability for XO to generate O_2^- has been known since 1968 [31], the capacity of this enzyme to participate in the immune response modulation, in contrast to defined intracellular pathogen killing, is recently recognized especially in regard to its crosstalk with NO function [32,33] and its role in mediating oxidative stress associated with intestinal and liver ischemia–reperfusion injuries typical of acute endotoxemia vascular shunting [34]. Many of these effects appear to be linked to the elaboration of cytokines such as tumor necrosis factor-α [12] as would follow from some form of bacterial toxin challenge. While models of proinflammatory stress using various delivery paradigms for LPS have been criticized as not truly being reflective of the pathophysiological time table of bacterial infection or sepsis, the use of LPS offers an opportunity to dissect components of the host response to this bacterial toxin in a very repeatable fashion. In particular, our use of the dual LPS challenge is rather unique in that few, if any other laboratories, study the response to LPS with regard to the impact of treatment on the tolerance response to the second challenge. Furthermore, very recent data suggest that acute, bolus-like LPS releases into the systemic circulation of septic patients may arise as a result of antibiotic therapy and its effect on bacterial membrane integrity. In these circumstances, clinical data indicate that patients experiencing this phenomenon are at greater risk for multiorgan failure and vascular collapse despite falling counts of pathogenic bacteria [35].

Although numerous strategies have been proposed to improve the clinical outcome of systemic Gram-negative sepsis, few have been more studied with equivocal conclusions than the use of GH as an adjunct therapy meant to preserve nitrogen balance and improve immune function. According to a given protocol, GH administration has been associated with both improvement [14,36,37], for example, as well as destabilization of organ function [15,38,39] leading to a significant rethinking of the appropriateness of GH therapy in some instances of illness. Some light on why this difference in opinion exists may reside in the results of Roelfsema et al. [40] who suggested that the metabolic outcome of subjects challenged with LPS may be related to the pattern of GH administration. Data from our laboratory align with this thought where the duration of GH treatment substantively affected when and to what extent the urea cycle and arginase activity, specifically, were decreased by GH treatment [17]. The effect of GH on arginase activity is regarded as a major point of impact through which GH might increase NO production because of the competition between the urea cycle and the NO synthase pathways for the common substrate arginine [41].

The very nature of the chemical reactivity of XO reaction products as well as NO causes several possibilities to develop through which processes either protective or destructive to an animal could develop. For example, increased localized production of NO in association with GH treatment could be viewed as a means of preserving blood flow and oxygenation to tissue beds that otherwise may have become ischemic in response to toxic shock. The increase in O_2^- production as well as UA commensurate with the increase in plasma XO activity could be viewed as providing a sink for excess NO scavenging further localizing NO activity, an enhanced production of the natural antioxidant UA, and increased oxidative reactivity of O_2^- and H_2O_2 needed for pathogen killing. Similarly, the converse of this has been applied to NO, that role of NO being a scavenger of O_2^- and hemoglobin being a scavenger of NO [42]. On the other hand, increased O_2^- production coordinately linked with increased NO production has been related to the formation of the highly reactive anion peroxynitrite ($ONOO^-$) under proper intracellular red-ox states that rapidly nitrates the phenolic ring structure of protein tyrosine residues and nitrosylates cysteine thiol groups, both of which alter regulatory functions of impacted proteins [43].

Few data exist that describe an effect of GH on the regulation of XO. Two early studies testing whether GH might affect XO activity were associated with observations that liver XO activity markedly increased in hypophysectomized rats although GH administration was without effect to lower this increase [44,45]. Even if there had been an effect of GH, the largely nonphysiological context of the panhypopituitarism induced via transauricular hypophysectomy with accompanying metabolic perturbations would make assessment of the results equivocal and problematic. The present paper is the first to describe the effects of GH treatment to impact the XO activity changes in plasma during immune challenge as modeled by LPS administration. In this regard we have identified XO as a potential major contributor to variations in clinical complications observed with the use of GH in subjects of ill health in its capacity to function outside of the confines of specialty cell populations such as neutrophils and macrophages.

GH has been found to enhance activities of major immune cell populations important to counteracting infectious organisms. GH was shown to augment macrophage activation [46] and increase O_2^- production in cultured neutrophils [47]. Clinically, GH administration to hypopituitary adults was associated with the restoration of the oxidative bursts of neutrophils [48], suggesting improved capacity for GH-treated subjects to fight invading pathogens. A recent report, however, suggests that complications in this effect of GH may reside in the development of microvascular pathologies where activated neutrophils sequester in and damage lung tissue [39] via the production (and presumably leakage) of reactive oxygen species. Where this specialized enhanced production of O_2^- in the face of GH administration is well recognized, few data exist on hormonal regulation of the other sources of O_2^- generation, namely, XO. The control of the O_2^- generation burst largely has been attributed to NAD(P)H oxidase events [49] although the participation of collateral pathways has been postulated [50]. However, the capacity for GH to increase O_2^- production in this manner may not be a consistent finding across a range of cell types. Most recently, Arnold and Weigent [51] demonstrated that both GH administration to, as well as over expression of GH by the EL4 T-cell lymphoma cell line resulted in a decreased O_2^- generation unassociated with either XO or NAD(P)H oxidase but rather through a select component of the P450 complex.

Bacterial infection, sepsis, and endotoxemia cumulatively disturb normal metabolic processes in a coordinated pattern of effects that are temporally orchestrated and connected [17]. The temporal connection is largely the process of an immune response cascade involving induction of proinflammatory cytokines, arachidonic acid mediators, compartmentalized NO production, and oxygen free radical and reactive nitrogen anion generation. Elucidation of pivotal control points in the biochemical pathways that converge to perturb organ function is a critical need in the construction of intervention strategies useful to remediate the extent of metabolic impairment and homeostatic compromise. To this end, a critical role in the development of LPS-related oxidative stress has been ascribed to XO activity as it has been identified as a major source of superoxide anion-driven intracellular damage in these stress situations.

The biological properties of E were exploited in the present study to serve as a probe to establish whether the observed effects of GH on XO and NO were of a character that would lend to modulation and pre-emptive intervention. In the present study, short-term administration of E prior to a combination of GH treatment and LPS challenge attenuated stimulatory effects of GH on the generation of NO and activity of XO. Historically, E has been associated with chemical events involving its role as a major endogenous, chain-breaking antioxidant intercollated into cell membranes [18]. In this compartment, it effectively decreases lipid peroxidation by scavenging membrane-soluble electrophilic nitrogen oxides [52] and by inhibiting the propagation step of lipid peroxidation [53]. More recently, however, newer functions of E have been identified in the immune response to stress. Among these newly recognized functions are decreased proinflammatory cytokine elaboration [54], alterations in the arachidonic acid cascade, NF-κB activation, protein kinase C activity, as well as newly defined influences on endogenous GH and prolactin secretion [18]. Thus, under the present circumstances, the fact that E blunted the measured responses implies that GH may largely upregulate proinflammatory states with a concurrent production of oxidizing and nitrating reactants.

The present study constitutes the first systematic clarification of XO as a critical immune response control point impacted by GH administration. Ascribing neither a positive nor a negative health impact of the observed results at this time, we have established that GH treatment prior to immune challenge augments XO activity as well as increases NO production after LPS challenge. Accordingly, it is how the body further processes the products of XO activity (O_2^-, H_2O_2, and UA, for example) and NO generated via the multiple isoforms of NO synthase that will determine whether tissue structures or the overall health of an animal is maintained or further perturbed.

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